

CLAIMS

- [1] A method of measuring a glycated amine, comprising the steps of:
adding a protease to a sample to degrade a glycated amine as an
5 analyte contained in the sample with the protease;
adding a fructosyl amino acid oxidase (FAOD) to the sample so that
the FAOD acts on the degradation product of the glycated amine, thereby
causing a redox reaction;
measuring the redox reaction; and
10 determining an amount of the glycated amine based on a result of the
measurement of the redox reaction,
wherein the method further comprises, prior to the degradation step
of adding the protease, a step of adding the FAOD to the sample to cause the
FAOD to act on a non-analyte glycated amine that is present in the sample
15 and different from the glycated amine as the analyte in order to remove an
influence of the non-analyte glycated amine, and
the redox reaction after the degradation step is caused by the FAOD
added prior to the degradation step.
- [2] The method according to claim 1, wherein the degradation step, the
20 step of causing the redox reaction, and the step of measuring the redox
reaction are performed at the same time.
- [3] The method according to claim 1, wherein the step of causing the
redox reaction is a step of causing the FAOD to act on the degradation
product of the glycated amine to generate hydrogen peroxide.
- 25 [4] The method according to claim 3, wherein the step of measuring the
redox reaction comprises a step of adding an oxidase and a substrate that
develops color by oxidation to the sample so that a reaction between the
generated hydrogen peroxide and the substrate is caused by the oxidase.
- [5] The method according to claim 4, wherein the degradation step, the
30 step of causing the redox reaction, and the step of measuring the redox

reaction are performed at the same time by adding the protease, the oxidase, and the substrate that develops color by oxidation to the sample at the same time after the step of causing the FAOD to act on the non-analyte glycosylated amine.

5 [6] The method according to claim 4, wherein the degradation step, the step of causing the redox reaction, and the step of measuring the redox reaction are performed at the same time after the step of causing the FAOD to act on the non-analyte glycosylated amine by adding the oxidase to the sample together with the FAOD prior to the degradation step and further adding the
10 protease and the substrate that develops color by oxidation to the sample at the same time.

[7] The method according to claim 4, wherein the degradation step, the step of causing the redox reaction, and the step of measuring the redox reaction are performed at the same time after the step of causing the FAOD
15 to act on the non-analyte glycosylated amine by adding the substrate that develops color by oxidation to the sample together with the FAOD prior to the degradation step and further adding the protease and the oxidase to the sample at the same time.

[8] The method according to claim 4, wherein the oxidase is peroxidase.

20 [9] The method according to claim 1, wherein the FAOD is an enzyme specific for a glycosylated α -amino group of an amino acid residue, an enzyme specific for a glycosylated side chain of an amino acid residue, or an enzyme specific for both a glycosylated α -amino group of an amino acid residue and a glycosylated side chain of an amino acid residue.

25 [10] The method according to claim 1, wherein the non-analyte glycosylated amine is a glycosylated amino acid.

[11] The method according to claim 1, wherein the glycosylated amine as the analyte is a glycosylated peptide or a glycosylated protein.

[12] The method according to claim 1, wherein the glycosylated amine as the
30 analyte is a glycosylated amine present in a blood cell.

[13] The method according to claim 1, wherein the glycated amine as the analyte is glycated hemoglobin.

[14] The method according to claim 1, wherein a tetrazolium compound further is added to the sample prior to the degradation step.

5 [15] The method according to claim 14, wherein the tetrazolium compound comprises 2-(4-iodophenyl)-3-(2,4-dinitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium or a salt thereof.

[16] The method according to claim 1, wherein a surfactant further is added to the sample prior to the degradation step.

10 [17] The method according to claim 16, wherein the surfactant is at least one surfactant selected from amphoteric surfactants, anionic surfactants, and cationic surfactants.

[18] A reagent kit to be used in the method according to claim 1, the reagent kit comprising a first reagent and a second reagent,

15 wherein the first reagent contains at least a fructosyl amino acid oxidase (FAOD),

the second reagent contains at least a protease, and

one of a peroxidase and a substrate that develops color by oxidation is contained in the first reagent whereas the other is contained in the second reagent, or both the peroxidase and the substrate are contained in the second reagent.

[19] The reagent kit according to claim 18, wherein the first reagent further contains a tetrazolium compound.

25 [20] The reagent kit according to claim 19, wherein the tetrazolium compound comprises 2-(4-iodophenyl)-3-(2,4-dinitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium or a salt thereof.

[21] The reagent kit according to claim 19, wherein the first reagent further contains a surfactant.